Litton BIONETICS

JJ25

MUTAGENICITY EVALUATION
OF
FDA 75-76
RIBOFLAVIN USP-FCC

FINAL REPORT

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20795

Mutagenic Evaluation of Compound FDA 75-76 (Riboflavin USP-FCC) Final report
7/77

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RIBOFLAVIN USP-FCC

FINAL REPORT

SUBMITTED TO

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DIVISION OF TOXICOLOGY
BUREAU OF FOODS
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Litton BIONETICS

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EVALUATION SUMMARY

The test compound, FDA 75-76, Riboflavin USP-FCC, did not exhibit mutagenic activity in any of the assays employed in these studies.

DATE: July, 1977

SPONSOR: U.S. Food and Drug Administration

SUBJECT: Evaluation of Test Compound: FDA 75-76, Riboflavin USP-FCC

I. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

II. MATERIALS

A. Test Compound

1. Date Received: October 29, 1976

2. Description: Yellow-gold powder

B. Indicator Microorganisms

The following strains of indicator microorganisms were used in the evaluation:

Yeast Strain: *Saccharomyces cerevisiae*, strain D4

Bacteria Strains: *Salmonella typhimurium*, strains TA-1535
TA-1537
TA-1538
TA-98
TA-100

C. Reaction Mixture

The following reaction mixture was employed in the activation tests:

Component	Final Concentration/ml
1. TPN (sodium salt)	4 µmoles
2. Glucose-6-phosphate	5 µmoles
3. Sodium phosphate (dibasic)	100 µmoles
4. $MgCl_2$	8 µmoles
5. KCl	33 µmoles
6. Homogenate fraction equivalent to 25 mg of wet tissue.	

D. Tissue Homogenates and Supernatants

The tissue homogenates and 9,000 x g supernatants were prepared from tissues of the following mammalian species: Mouse - ICR random bred adult males; rat - Sprague-Dawley adult males; and monkey - Macaca mulatta adult males.

E. Positive Control Compounds

Table 1 lists chemicals for positive controls in the direct and activation assays.

TABLE 1

POSITIVE CONTROLS USED IN DIRECT AND ACTIVATION ASSAYS

Assay	Chemical[a]	Solvent	Probable Mutagenic Specificity
Nonactivation	Methylnitrosoguanidine	Water or saline	BPS[b]
	Ethylmethanesulfonate	Water or saline	BPS[b]
	2-Nitrofluorene	Dimethylsulfoxide[c]	FS[b]
	Quinacrine mustard	Water or saline	FS[b]
Activation	Dimethylnitrosamine	Water or saline	BPS[b]
	2-Acetylaminofluorene	Dimethylsulfoxide[c]	FS[b]
	8-Aminoquinoline	Dimethylsulfoxide[c]	FS[b]
	2-Aminoanthracene	Dimethylsulfoxide[c]	BPS[b]

[a] Concentrations given in the Results Section
[b] BPS = base-pair substitution; FS = frameshift
[c] Previously shown to be non-mutagenic

III. METHODS

A. Toxicity

The solubility, toxicity and doses for the test chemical were determined prior to screening.

The test chemical was tested for toxicity against specific indicator strains over a range of doses to determine the 50% survival dose. Bacteria were tested in phosphate buffer, pH 7.4, for one hour at 37°C on a shaker. Yeasts were tested in phosphate buffer, pH 7.4, for four hours at 30°C on a shaker. The 50% survival concentrations and the 1/4 and 1/2 50% doses calculated.

If no toxicity was obtained for the chemical with a given strain, then a maximum dose of 5% (w/v) was used.

Unless otherwise specified, the doses calculated for the tests in buffer were applied to the activation tests. The solubility of the test chemical under treatment conditions is stated in the Results Section.

B. Plate Tests (Overlay Method)

Approximately 10^8 cells from an overnight culture of each indicator strain were added to test tubes containing 2.0 ml of molten agar supplemented with biotin and a trace of histidine. For nonactivation tests, the three dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests 0.5 ml of a 9,000 x g tissue supernatant and required cofactors (core reaction mixture) were added to the overlay tubes. Three dose levels of the test chemical were added to the appropriate tubes, which were then mixed and the contents poured over the surface of a minimal agar (selective medium) plate and allowed to solidify. The plates were incubated for 48 to 72 hours at 37°C, and scored for the number of colonies growing on each plate. The concentrations of all chemicals are given in the Results Section. Positive and solvent controls using positive compounds that are active directly and those that require metabolic activation were run with each assay.

C. Suspension Tests

1. Nonactivation

Bacteria and yeast cultures of the indicator organisms were grown in complete broth, washed and resuspended in 0.9% saline to densities of 1×10^{10} cells/ml and 5×10^9 cells/ml, respectively. This constituted the working stock for tests of a group of test chemicals and their respective controls. Tests were conducted in plastic, 24-well tissue culture plates (Linbro). Cells plus appropriate volume(s) of the test chemical were added to the wells to give a final volume of 1.5 ml. The solvent replaced the test chemical in the negative controls. Treatment was at 30°C for four hours for yeast tests and at 37°C for one hour for bacterial tests. All flasks were shaken during treatment. Following treatment, the plates were set on ice. Aliquots of cells were removed, diluted in sterile saline (4°C) and plated on the appropriate complete media. Undiluted samples from flasks containing the bacteria were plated on minimal selective medium in reversion experiments. Samples from a 10^{-1} dilution of treated cells were plated on the selected media for enumeration of gene conversion with strain D4. Bacterial plates were scored after incubation for 48 hours at 37°C. The yeast plates were incubated at 30°C for 3-5 days before scoring.

2. Activation

Bacteria and yeast cells were grown and prepared as described in the nonactivation tests. Measured amounts of the test and control chemicals plus 0.25 ml of the stock-cell suspension were added to wells of the Linbro plate containing the appropriate tissue fraction and reaction mixture. All flasks (bacteria and yeast) were incubated at 37°C with shaking. The treatment times as well as the dilutions, plating procedures and scoring of the plates were the same as described for nonactivation tests.

D. Preparation of Tissue Homogenates and 9,000 x g Cell Fractions

Male animals (except monkeys) sufficient to provide the necessary quantities of tissues were killed by cranial blow, decapitated and bled. Monkey tissues were obtained from freshly killed and bled male rhesus monkeys. Organs were immediately dissected from the animals using aseptic techniques and placed in ice-cold 0.15M KCl. Upon collection of the desired quantity of organs, they were washed twice with fresh KCl and completely homogenized with a motor-driven homogenizing unit at 4°C. The whole organ homogenate obtained from this step was divided into two samples. One sample was frozen at -80°C and the other was centrifuged for 20 minutes at 9,000 x g in a refrigerated centrifuge. The supernatant from the centrifuged sample was retained and frozen at -80°C. These two frozen samples were used for the activation studies. Protein and P-448 determinations were made for each lot of homogenate.

E. Data Recording and Reporting

1. Plate test assays

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were entered into a computer program designed to print out all data by test. The data are presented as revertants per plate for each indicator strain employed in the assay. The positive and solvent controls are provided as reference points.

2. Suspension assays

Following the specified incubation periods all population plates were scored by an automatic colony counter and the results from each plate of a set were recorded, in ink, on data processing forms. All minimal or other types of selective media plates were hand scored and the results recorded along with the respective population data. Other relevant experimental data were recorded on experimental definition forms. For bacteria strains the number of colonies recorded from either the population or selective plates represents that number in 1 ml of test suspension plated. The numbers recorded for the yeast strain D4 represent the number in 0.5 ml of test suspension plated. The data were then processed and printed from a computer program. All raw data sheets are dated and signed by the responsible technician.

IV. RESULTS SECTION

A. Solubility Properties of the Test Compound

1. Name or code designation of the test compound: FDA 75-76, Riboflavin USP-FCC

2. Test solvent: * DMSO

3. Solubility of the test compound under treatment conditions: Soluble

4. Additional comments: Yellow-gold powder

B. Toxicity and Dosage Determinations for the Test Compound

1. Test date for toxicity determination: December 22, 1976

2. The 50% survival level was determined for bacteria and yeast indicator organisms by conducting survival curves with the test compound at the following concentrations:

Percent Concentration (w/v or v/v)

5.0
0.5
0.05
0.005
0.0005

3. Concentrations of the test compound used in the mutagenicity tests:

	Percent Concentration	
Test Doses	Bacteria	Yeast
1/4 50% Survival	1.25	1.25
1/2 50% Survival	2.50	2.50
50% Survival	5.00	5.00

*The concentration of solvent was equal to the highest volume of test material added.

C. Plate Test Results

The plate test results are summarized in the following table. The values presented in this table are the number of revertants per plate.

D. Suspension Assay Results

The suspension test results for the test compound are summarized in the tables following the plate test summary. The values presented in these tables are the calculated mutation frequencies for each control and experimental test point. The first table of the suspension set presents the results for the nonactivation assays, and the second table through the fourth table of the suspension set presents the results for the activation assays. A listing of computer codes and abbreviations is included for reference. Tabulation of all raw data is provided in the Appendix.

SUMMARY OF TEST RESULTS

PLATE TESTS

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: 000083885

B. TEST DATE: APRIL 8, 1977

TEST		SPECIES	TISSUE	REVERTANTS PER PLATE									
				TA-1535		TA-1537		TA-1538		TA-98		TA-100	
				1	2	1	2	1	2	1	2	1	2
1. NON-ACTIVATION													
SOLVENT CONTROL*		---	---	11	18	11	16	10	19	37	51	270	263
POSITIVE CONTROL**		---	---	>1000	>1000	>1000	>1000	773	823	>1000	>1000	800	728
TEST	5.00000 %	---	---	17	10	15	17	13	15	41	39	131	150
	2.50000 %	---	---	16	18	14	12	15	11	36	42	237	216
	1.25000 %	---	---	12	17	19	11	14	16	31	38	288	201
2. ACTIVATION													
SOLVENT CONTROL*		MOUSE	LIVER	25	22	18	10	30	22	35	39	129	148
		RAT	LIVER	34	27	21	19	18	20	37	40	128	155
		MONKEY	LIVER	25	26	13	17	18	17	42	39	117	148
POSITIVE CONTROL***		MOUSE	LIVER	848	>1000	207	766	>1000	>1000	>1000	>1000	797	871
		RAT	LIVER	353	388	621	434	>1000	>1000	>1000	>1000	>1000	>1000
		MONKEY	LIVER	204	319	511	215	665	871	>1000	>1000	>1000	>1000
TEST	5.00000 %	MOUSE	LIVER	16	28	15	9	10	13	37	35	130	126
	2.50000 %	MOUSE	LIVER	25	19	11	10	17	19	48	45	124	128
	1.25000 %	MOUSE	LIVER	27	24	15	13	11	12	46	40	156	179
	5.00000 %	RAT	LIVER	24	27	11	15	15	11	46	40	125	126
	2.50000 %	RAT	LIVER	19	17	17	15	15	12	40	38	126	119
	1.25000 %	RAT	LIVER	26	28	17	13	17	20	32	54	143	167
	5.00000 %	MONKEY	LIVER	22	37	16	11	18	19	45	53	127	130
	2.50000 %	MONKEY	LIVER	24	23	24	13	19	10	51	54	120	176
	1.25000 %	MONKEY	LIVER	28	27	15	12	14	14	37	36	130	170

* NON-ACTIVATION ASSAYS CONSIST OF THE CELLS PLUS THE TEST COMPOUND VEHICLE (SOLVENT). FOR ACTIVATION ASSAYS, THE OVERLAY CONTAINS THE ACTIVATION SYSTEM PLUS THE TEST COMPOUND VEHICLE.

** TA-1535 MNNG 2 UG/PLATE
TA-1537 QM 20 UG/PLATE
TA-1538 NF 100 UG/PLATE
TA-98 NF 100 UG/PLATE
TA-100 MNNG 2 UG/PLATE

*** TA-1535 ANTH 100 UG/PLATE
TA-1537 AMQ 100 UG/PLATE
TA-1538 AAF 100 UG/PLATE
TA-98 AAF 100 UG/PLATE
TA-100 ANTH 100 UG/PLATE

NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS(UL) OR MICROGRAMS(UG) PER PLATE.

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 07/27/77

NONACTIVATION COMPOUND 000083885

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	
NAN		101.71	16.77	8.45	5.46	27.61	18.25	11.73	CONTROLS
NAP		2775.23	390.27	611.11	163.30	154.04	160.82	124.93	
NA1		135.34	9.49	2.54	3.54	13.34	14.94	6.96	TEST DATA
NA2		95.53	12.66	2.52	2.86	19.85	17.52	9.77	
NA3		110.79	11.50	4.70	4.14	13.37	16.06	7.28	

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 07/27/77

SPECIES ICRFLO/MOUSE COMPOUND 000083885

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	
ACT	A+C	74.92	4.47	6.87	10.74	9.69	1.42	0.87	NEGATIVE CONTROLS
ACT	A-C	95.34	2.95	7.88	7.24	14.07	2.23	1.78	
ACT	ALI	75.23	5.66	7.30	10.51	30.17	8.45	3.69	
ACT	ALU	76.77	8.90	7.14	11.33	20.99	6.55	4.68	
ACT	PLI	203.57	150.23	94.83	668.98	135.88	139.35	75.03	POSITIVE CONTROLS
ACT	PLU	78.39	14.54	5.79	18.52	56.13	8.25	2.71	
ACT	LI1	88.24	3.70	2.78	8.33	3.32	3.98	2.93	TEST COMPOUND
ACT	LI2	58.09	4.39	3.85	7.39	8.02	4.17	3.88	
ACT	LI3	62.55	5.47	2.36	7.73	24.81	6.14	5.18	
ACT	LU1	62.31	1.59	4.81	8.02	10.07	6.01	4.46	
ACT	LU2	69.97	8.62	3.39	11.85	11.92	5.81	4.57	
ACT	LU3	70.04	3.98	3.46	7.03	11.51	7.00	4.96	

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 07/27/77

SPECIES SPROAW/RAT COMPOUND 000083885

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	
ACT	A+C	55.00	8.04	7.37	5.76	8.23	13.27	8.24	NEGATIVE CONTROLS
ACT	A-C	43.54	6.10	1.71	7.75	5.63	22.68	9.82	
ACT	ALI	117.84	10.14	3.54	7.07	40.57	27.77	15.41	
ACT	ALU	120.43	12.87	4.77	7.46	35.33	24.60	11.70	
ACT	PLI	302.83	189.41	62.91	126.90	235.81	84.59	45.78	POSITIVE CONTROLS
ACT	PLU	66.33	9.42	2.79	8.30	173.22	16.03	11.02	
ACT	LI1	65.19	6.91	2.72	5.24	13.16	12.89	7.59	TEST COMPOUND
ACT	LI2	88.74	13.49	3.31	7.53	9.16	12.13	7.19	
ACT	LI3	80.62	12.93	4.89	6.80	9.17	14.41	6.98	
ACT	LU1	52.77	9.88	4.17	6.91	9.28	14.43	5.41	
ACT	LU2	91.27	10.00	2.95	6.25	8.03	19.97	7.83	
ACT	LU3	99.65	5.70	2.60	6.94	7.61	22.24	9.13	

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 07/27/77

SPECIES RHESUS/MONKEY COMPOUND 000083885

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	
ACT	A+C	71.22	15.03	3.16	1.78	7.97	20.69	8.30	NEGATIVE CONTROLS
ACT	A-C	24.59	9.17	4.03	2.76	8.66	17.36	7.70	
ACT	ALI	80.81	13.76	5.68	5.01	31.01	15.76	9.19	
ACT	ALU	74.62	17.42	4.33	8.42	16.75	20.62	8.94	
ACT	PLI	173.50	90.35	57.70	114.16	672.39	88.34	62.48	POSITIVE CONTROLS
ACT	PLU	73.62	12.79	2.79	5.01	8.68	16.17	11.98	
ACT	LI1	71.92	7.39	3.61	3.13	4.80	19.37	10.91	TEST COMPOUND
ACT	LI2	82.50	13.83	2.89	3.65	5.51	20.77	10.68	
ACT	LI3	73.83	15.01	4.39	4.68	6.61	16.12	11.01	
ACT	LU1	64.40	7.26	3.36	4.18	4.98	15.35	9.85	
ACT	LU2	68.21	13.29	3.55	5.59	5.02	14.73	10.11	
ACT	LU3	83.42	9.23	2.05	4.91	4.57	15.31	9.72	

DATA TABLE TERMS AND ABBREVIATIONS

ABBREVIATION OR TERM	DEFINITION OR EXPLANATION
COMPOUND	Client designated compound number appears in this column.
TEST CODES	NAN = Nonactivation: Solvent Control NAP = Nonactivation: Positive Control NA1 = Nonactivation: Test Compound Dose 1 NA2, etc. = Reflects the other dose level(s) A+C = Negative Chemical Control for ACP A-C = Activation: Solvent Control ALI or A+T = Activation: Homogenate Control (Liver) ALU or A+T = Activation: Homogenate Control (Lung) ACP = Activation: Positive Control ACT = Activation Test LI = Liver Tissue Activation Fraction LU = Lung Tissue Activation Fraction KI = Kidney Tissue Activation Fraction TE = Testes Tissue Activation Fraction 1,2, etc. = Dose Levels
CONCENTRATION	All test compound dose levels are expressed as a whole number followed by an exponent (negative) identified by the appropriate units. Example: 0025-2PCT = 0.25 percent concentration
POPU	Total number of viable cells in the plating sample raised to some exponent printed directly below the abbreviation (i.e., EP + 6 = x 10^6).
MUT 1	Total number of mutants or convertants obtained from the sample plated raised to some exponent printed directly below the abbreviation (i.e., EP + 0 = 10^0). For strain D4, MUT 1 represents the number of ADE+ convertants.
MUT 2	Only used for strain D4 and represents the number of TRY+ convertants in the plated sample.
FREQ 1	The calculated mutation or gene conversion frequency times the negative exponent written directly below. For strain D4, FREQ 1 represents the ADE+ value.
FREQ 2	Only used for strain D4 and represents the TRY+ conversion frequency.
CONTAM	Presence of contamination on any plates.

DATA TABLE TERMS AND ABBREVIATIONS (continued)

ABBREVIATION OR TERM	DEFINITION OR EXPLANATION
AAF	2-Acetylaminofluorene
DMSO	Dimethylsulfoxide
DMN	Dimethylnitrosamine
EMS	Ethylmethanesulfonate
QM	Quinacrine Mustard
NF	Nitrofluorene
ANTH	2-Amino Anthracene
AMQ	8-Amino Quinoline
SPECIES	Animal Strains
SPRDAW	Sprague Dawley Rats
ICRFLO	Flow ICR Random Bred Mice
RHESUS	Rhesus Monkey (Macaca mulatta)
MIXEDB	Dog, Mixed Breed
NEWZEA	New Zealand White Rabbit
UG	Microgram
UM	Micromole
ADE	Adenine
TRY	Tryptophan

V. INTERPRETATION OF RESULTS AND CONCLUSIONS

The test compound, FDA 75-76, Riboflavin USP-FCC, was evaluated for genetic activity in a series of *in vitro* microbial assays with and without metabolic activation. The following results were obtained:

A. Salmonella typhimurium

1. Plate tests

The results of these tests were negative.

2. Nonactivation suspension tests

The results of these tests were negative.

3. Activation suspension tests

The results of these tests were negative.

B. Saccharomyces cerevisiae

1. Nonactivation suspension tests

The results of these tests were negative.

2. Activation suspension tests

The results of these tests were negative.

C. Conclusions

The test compound, FDA 75-76, Riboflavin USP-FCC, did not exhibit mutagenic activity in any of the assays employed in these studies.

Submitted by:

7/29/77

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Director
Department of Molecular Toxicology

Reviewed by:

7/29/77

Robert J. Weir, Ph.D. Date
Vice President

VI. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS

Plate test data consist of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Because the test chemical and cells are incubated in the overlay for 2-3 days, and a few cell divisions occur during the incubation period, the test is semiquantitative in nature. Although these features of the assay reduce the quantitation of results, they provide certain advantages not contained in a quantitative suspension test.

- The small number of cell divisions permits potential mutagnes to act on replicating DNA which is often more sensitive than non-replicating DNA.
- The combined incubation of the compound and the cells in the overlay permit constant exposure of the indicator cells for 2-3 days.

A. Surviving Populations

Plate test procedures do not permit exact quantitation of the number of cells surviving chemical treatment. At low concentrations of the test chemical, the surviving population on the treatment plates is essentially the same as the negative control plate. At high concentrations, the surviving population is usually reduced by some fraction. Our protocol normally employs dose levels that are selected such that the highest dose will show slight toxicity (as determined by subjective criteria) and several doses ranging down 1 to 2 logs lower.

B. Dose Response Phenomena

The demonstration of dose-related increases in mutant counts is an important criterion in establishing mutagenicity. Factors which may modify dose response results for a mutagen would be the selection of doses that are too low (usually mutagenicity and toxicity are related). If the highest dose is far lower than a toxic concentration, no increases may be observed over the dose range selected. Conversely, if the lowest dose employed is highly cytotoxic, the test chemical may kill any mutants that are induced and the compound will not appear to be mutagenic.

C. Control Tests

Positive and negative control assays are conducted with each experiment and consist of direct acting mutagens for nonactivation assays and mutagens that require metabolic biotransformation in activation assays. Negative controls consist of the test compound solvent in the overlay agar with the other essential components. The negative control plate for each strain gives a reference point to which the test data are compared. The positive control assay is conducted to demonstrate that the test systems are functional with known mutagens.

D. Evaluation Criteria for Ames Assay

Because the procedures used to evaluate the mutagenicity of the test chemical are semiquantitative, the criteria used to determine positive effects are inherently subjective and are based primarily on a historical data base. Most data sets are evaluated using the following criteria:

1. Strains TA-1535, TA-1537, and TA-1538

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the lowest increase equal to twice the solvent control value is considered to be mutagenic.

2. Strains TA-98, TA-100, and D4

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the highest increase equal to twice the solvent control value for TA-100 and two to three times the solvent control value for strains TA-98 and D4 is considered to be mutagenic. For these strains, the dose response increase should start at approximately the solvent control values.

3. Pattern

Because TA-1535 and TA-100 were both derived from the same parental strain (G-46) and because TA-1538 and TA-98 were both derived from the same parental strain (D3052), there is a buil-in redundancy in the microbial assay. In general the two strains of a set respond to the same mutagen and such a pattern is sought. It is also anticipated that if a given strain, e.g. TA-1537, responds to a mutagen in nonactivation tests it will generally do so in activation tests. (The converse of this relationship is not expected.) While similar response patterns are not required for all mutagens, they can be used to enhance the reliability of an evaluation decision.

4. Reproducibility

If a chemical produces a response in a single test that cannot be reproduced in one or more additional runs, the initial positive test data loses significance.

The preceding criteria are not absolute and other extenuating factors may enter into a final evaluation decision. However, these criteria are applied to the majority of situations and are presented to aid those individuals not familiar with this procedure. As the data base is increased, the criteria for evaluation can be more firmly established.

VII. EXPLANATION OF EVALUATION PROCEDURES FOR SUSPENSION ASSAYS

Data obtained from mutagenicity tests are evaluated on a test by test basis followed by an examination of the total response pattern using all the data. To facilitate this type of evaluation, we have prepared two separate formats in which data are processed. The first is the Compound Summary Backup Detail Sheet, which details the essential raw data from each experiment showing surviving population counts, total mutant or convertant counts, as well as, calculated mutation frequencies. This format permits close examination of each set of test data. The following considerations are part of any assessment.

A. Surviving Population Counts

A certain level of chemically-induced toxicity is anticipated, but occasionally isolated tests or groups of tests show very low (<25%) survival compared to the tissue controls. Such isolated decreases may result from improper dilution procedures or defective growth media and decrease confidence in the calculated mutation frequencies especially if the total mutant counts appear unaffected. Data of this type are generally unacceptable and these experiments are routinely repeated at a lower dose level to reduce killing and increase confidence in the nature of the response.

B. Total Mutant Counts

For nonmutagens, the mutant/surviving population ratio should be roughly equivalent for each test point in a given experiment. If the cell number drops in response to killing, the mutant number should decrease proportionately. A mutagenic chemical, however, will produce an altered mutant/surviving population ratio. Mutant numbers as well as calculated frequencies are compared to the negative control data. In certain instances, the mutant frequencies will increase with little or no change in the absolute number of mutants especially where the test chemical is toxic. Data of this type, although not necessarily aberrant, or even rare, must be viewed with special care to ensure that the increased frequencies were not the result of selective toxicity of the test chemical for the his cells. This phenomenon, referred to as selection, can lead to erroneous conclusions. Thus we attempt to keep the surviving population of cells high and look for positive responses that show increases in both numbers of mutants and mutation frequencies. Again, occasional isolated fluctuations in mutant counts are found that can be attributed to improper pipetting or media contamination. These fluctuations are usually easy to identify by inspection of the other data points in the experiment which will be negative.

C. Dose Response Phenomena

Dose-related increases in mutants and mutation frequencies are the most convincing data to have in assessing mutagenic activity of chemicals. In some cases, however, dose-related increases are not observed for mutagens. This depends considerably on the dose levels selected. The figure on the following page illustrates how one might obtain various types of dose-related responses by a mutagen based solely on dose selection. It also emphasizes the need to keep dose levels within a relatively low range of toxicity so that data are consistently on the uphill side of the hypothetical curve.

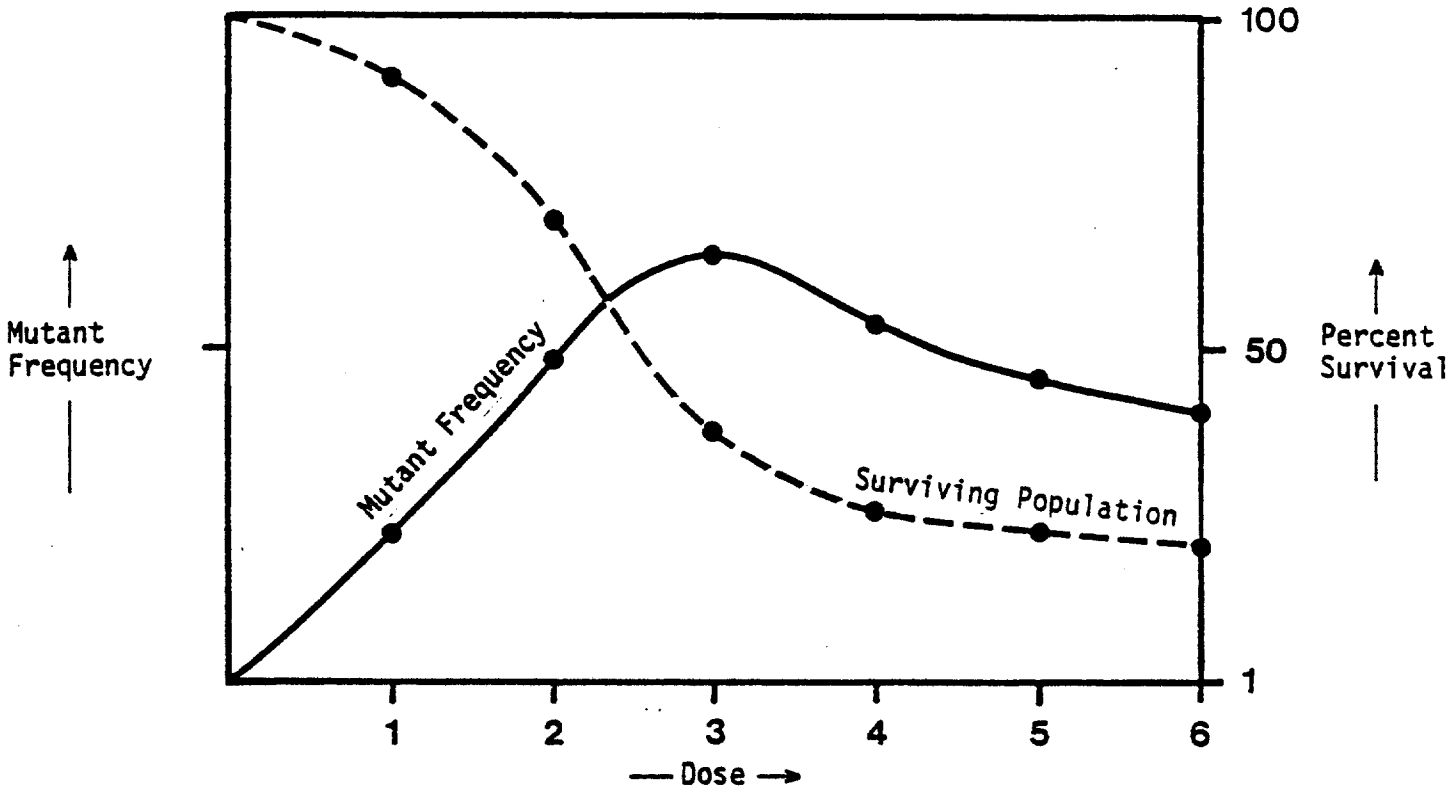
D. Control Tests

Positive and negative control tests are conducted with each experiment and consist of direct acting positive agents for nonactivation assays and chemicals that require metabolic transformation for activation assays. In nonactivation assays, the NAN control contain the test chemical solvent plus cells, but no chemical, and is used as a reference to assess the level of response obtained in the various tests. It is not possible at this time to put precise cut-off points where negative responses become positive responses. A statistical component for our computer program is under development and will be included when available. Positive controls are only used as relative reference points and to demonstrate that the system is functioning with known mutagens. In activation assays, three types of negative controls are run: (1) A solvent control minus the chemical and minus the activation system (A-C); (2) a control plus the positive control chemical minus the activation system (A+C); and (3) a control containing the activation system and the test chemical solvent (ALI or ALU). All three controls are used collectively to assess the level of response in the various activation tests. A chemical may appear positive when compared to an A-C control but not when compared to an A+T control. The value of each of the above controls with respect to their weight in evaluation is ALI or ALU > A-C > A+C.

The other data format is the Compound Frequency Summary Report sheet in which all the calculated frequencies obtained for a given compound are displayed in a table. This format permits an overview of all data. The points form a matrix of information that should present a consistent pattern. Nonmutagens should produce a matrix with data frequencies clustered around the negative control values. Occasional random high or low fluctuations are not uncommon and seldom indicate true genetic activity. Mutagenic chemicals should, on the other hand, produce a set of consistent responses that demonstrate a logical pattern. The patterns depend on the mutagenic specificity of the chemical but can be easily recognized in the Compound Frequency Summary Report format.

These mutagenicity assays are designed to optimize the probability of recognizing mutagens from nonmutagens and, in most cases, they work well. Occasionally, the data points are such that a definitive conclusion cannot be made without additional data.

HYPOTHETICAL MUTATION AND TOXICITY KINETICS



HYPOTHETICAL EXPERIMENT	OBSERVED DOSE RESPONSE
(1) Dose levels 1,2 & 3 were used	A typical positive dose response set of data would be obtained.
(2) Dose levels 2, 3 & 4 were used	The intermediate dose level shows a higher mutation frequency than both the low dose and the high dose.
(3) Dose levels 3, 4 & 5 were used	Here an inverted dose response would be observed with the highest dose level showing the lowest response.

APPENDIX

Tabulation of Data

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 636504 DETECTOR TA100 SPECIES / DATE - 07/27/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	NAN		SOLVENT	0351	0357	101.71	0
	NAP		EMS 0.066%	0218	6050	2775.23	0
000083885	NA1		0005-0 PCT.	0249	0337	135.34	0
000083885	NA2		0025-1 PCT.	0514	0491	95.53	0
000083885	NA3		0125-2 PCT.	0343	0380	110.79	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 636501 DETECTOR TA1535 SPECIES / DATE - 07/27/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	NAN		SOLVENT	0471	0079	16.77	0
	NAP		EMS 0.2%	0822	3208	390.27	0
000083885	NA1		0005-0 PCT.	0527	0050	9.49	0
000083885	NA2		0025-1 PCT.	0545	0069	12.66	0
000083885	NA3		0125-2 PCT.	0965	0111	11.50	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 636201 DETECTOR TA1537 SPECIES / DATE - 07/27/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	NAN		SOLVENT	0213	0018	8.45	0
	NAP		QM 13 UG/ML	0063	0385	611.11	0
000083885	NA1		0005-0 PCT.	0668	0017	2.54	0
000083885	NA2		0025-1 PCT.	0675	0017	2.52	0
000083885	NA3		0125-2 PCT.	0447	0021	4.70	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 705306 DETECTOR TA1538 SPECIES / DATE - 07/27/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	NAN		SOLVENT	0403	0022	5.46	0
	NAP		NF 667 UG/ML	0376	0614	163.30	0
000083885	NA1		0005-0 PCT.	0311	0011	3.54	0
000083885	NA2		0025-1 PCT.	0419	0012	2.86	0
000083885	NA3		0125-2 PCT.	0507	0021	4.14	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 636503 DETECTOR TA98 SPECIES / DATE - 07/27/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	NAN		SOLVENT	1043	0288	27.61	0
	NAP		NF 667 UG/ML	0890	1371	154.04	0
000083885	NA1		0005-0 PCT.	1192	0159	13.34	0
000083885	NA2		0025-1 PCT.	1199	0238	19.85	0
000083885	NA3		0125-2 PCT.	1802	0241	13.37	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 702606 DETECTOR 0000D4 SPECIES / DATE - 07/27/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	NAN		SOLVENT	0537	0098	0063	18.25	11.73	0
	NAP		EMS 1.0 %	0365	0587	0456	160.82	124.93	0
000083885	NA1		0005-0 PCT.	0589	0088	0041	14.94	6.96	0
000083885	NA2		0025-1 PCT.	0645	0113	0063	17.52	9.77	0
000083885	NA3		0125-2 PCT.	0604	0097	0044	16.06	7.28	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 704905 DETECTOR TA100 SPECIES ICRFLO/MOUSE DATE - 07/27/77

COMPOUND	TEST	OHG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	0638	0478	74.92	0
	A-C		SOLVENT	0579	0552	95.34	0
	ALI		TISSUE	0662	0498	75.23	0
	ALU		TISSUE	0637	0489	76.77	0
	ACP	LI	DMN 90 UM/ML	0841	1712	203.57	0
	ACP	LU	DMN 90 UM/NL	0782	0613	78.39	0
000083885	ACT	LI1	0005-0 PCT.	0544	0480	88.24	0
000083885	ACT	LI2	0025-1 PCT.	0711	0413	58.09	0
000083885	ACT	LI3	0125-2 PCT.	0533	0440	82.55	0
000083885	ACT	LU1	0005-0 PCT.	0674	0420	62.31	0
000083885	ACT	LU2	0025-1 PCT.	0686	0480	69.97	0
000083885	ACT	LU3	0125-2 PCT.	0711	0498	70.04	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 701201 DETECTOR TA1535 SPECIES ICRFLO/MOUSE DATE - 07/27/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	0470	0021	4.47	0
	A-C		SOLVENT	0475	0014	2.95	0
	ALI		TISSUE	0512	0029	5.66	0
	ALU		TISSUE	0517	0046	8.90	0
	ACP	LI	DMN 90 UM/ML	0444	0667	150.23	0
	ACP	LU	DMN 90 UM/ML	0454	0066	14.54	0
000083885	ACT	LI1	0005-0 PCT.	0459	0017	3.70	0
000083885	ACT	LI2	0025-1 PCT.	0501	0022	4.39	0
000083885	ACT	LI3	0125-2 PCT.	0457	0025	5.47	0
000083885	ACT	LU1	0005-0 PCT.	0439	0007	1.59	0
000083885	ACT	LU2	0025-1 PCT.	0487	0042	8.62	0
000083885	ACT	LU3	0125-2 PCT.	0503	0020	3.98	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 701803 DETECTOR TA1537 SPECIES ICRFLO/MOUSE DATE - 07/27/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		AMQ 333 UG/ML	0553	0038	6.87	0
	A-C		SOLVENT	0520	0041	7.88	0
	ALI		TISSUE	0712	0052	7.30	0
	ALU		TISSUE	0518	0037	7.14	0
	ACP	LI	AMQ 333 UG/ML	0329	0312	94.83	0
	ACP	LU	AMQ 333 UG/ML	0622	0036	5.79	0
000083885	ACT	LI1	0005-0 PCT.	0468	0013	2.78	0
000083885	ACT	LI2	0025-1 PCT.	0546	0021	3.85	0
000083885	ACT	LI3	0125-2 PCT.	0509	0012	2.36	0
000083885	ACT	LU1	0005-0 PCT.	0561	0027	4.81	0
000083885	ACT	LU2	0025-1 PCT.	0502	0017	3.39	0
000083885	ACT	LU3	0125-2 PCT.	0520	0018	3.46	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 708893 DETECTOR TA1538 SPECIES ICRFLO/MOUSE DATE - 07/27/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0633	0068	10.74	0
	A-C		SOLVENT	0898	0065	7.24	0
	ALI		TISSUE	0590	0062	10.51	0
	ALU		TISSUE	0547	0062	11.33	0
	ACP	LI	ANTH 67 UG/ML	0332	2221	668.98	0
	ACP	LU	ANTH 67 UG/ML	0583	0108	18.52	0
000083885	ACT	LI1	0005-0 PCT.	0552	0046	8.33	0
000083885	ACT	LI2	0025-1 PCT.	0528	0039	7.39	0
000083885	ACT	LI3	0125-2 PCT.	0569	0044	7.73	0
000083885	ACT	LU1	0005-0 PCT.	0673	0054	8.02	0
000083885	ACT	LU2	0025-1 PCT.	0439	0052	11.85	0
000083885	ACT	LU3	0125-2 PCT.	0512	0036	7.03	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 700301 DETECTOR TA98 SPECIES ICRFLO/MOUSE DATE - 07/27/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0609	0059	9.69	0
	A-C		SOLVENT	0533	0075	14.07	0
	ALI		TISSUE	0517	0156	30.17	0
	ALU		TISSUE	0705	0148	20.99	0
	ACP	LI	ANTH 67 UG/ML	1285	1746	135.88	0
	ACP	LU	ANTH 67 UG/ML	0718	0403	56.13	0
000083885	ACT	LI1	0005-0 PCT.	0784	0026	3.32	0
000083885	ACT	LI2	0025-1 PCT.	0648	0052	8.02	0
000083885	ACT	LI3	0125-2 PCT.	0270	0067	24.81	0
000083885	ACT	LU1	0005-0 PCT.	0685	0069	10.07	0
000083885	ACT	LU2	0025-1 PCT.	0621	0074	11.92	0
000083885	ACT	LU3	0125-2 PCT.	0912	0105	11.51	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 702605 DETECTOR 0000D4 SPECIES ICRFLO/MOUSE DATE - 07/27/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	1271	0018	0011	1.42	0.87	0
	A-C		SOLVENT	1569	0035	0028	2.23	1.78	0
	ALI		TISSUE	1030	0087	0038	8.45	3.69	0
	ALU		TISSUE	1283	0084	0060	6.55	4.68	0
	ACP	LI	DMN 90 UM/ML	0737	1027	0553	139.35	75.03	0
	ACP	LU	DMN 90 UM/ML	0958	0079	0026	8.25	2.71	0
000083885	ACT	LI1	0005-0 PCT.	1810	0072	0053	3.98	2.93	0
000083885	ACT	LI2	0025-1 PCT.	2036	0085	0079	4.17	3.88	0
000083885	ACT	LI3	0125-2 PCT.	1449	0089	0075	6.14	5.18	0
000083885	ACT	LU1	0005-0 PCT.	1615	0097	0072	6.01	4.46	0
000083885	ACT	LU2	0025-1 PCT.	1618	0094	0074	5.81	4.57	0
000083885	ACT	LU3	0125-2 PCT.	1271	0089	0063	7.00	4.96	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 701804 DETECTOR TA100 SPECIES SPRDAW/RAT DATE - 07/27/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	1300	0715	55.00	0
	A-C		SOLVENT	1091	0475	43.54	0
	ALI		TISSUE	0566	0667	117.84	0
	ALU		TISSUE	0460	0554	120.43	0
	ACP	LI	DMN 90 UM/ML	0494	1496	302.83	0
	ACP	LU	DMN 90 UM/ML	0888	0589	66.33	0
000083885	ACT	LI1	0005-0 PCT.	0790	0515	65.19	0
000083885	ACT	LI2	0025-1 PCT.	0906	0804	88.74	0
000083885	ACT	LI3	0125-2 PCT.	1027	0828	80.62	0
000083885	ACT	LU1	0005-0 PCT.	0902	0476	52.77	0
000083885	ACT	LU2	0025-1 PCT.	0825	0753	91.27	0
000083885	ACT	LU3	0125-2 PCT.	0867	0864	99.65	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 702701 DETECTOR TA1535 SPECIES SPRDAW/RAT DATE - 07/27/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		DMN 90 UM/ML	0224	0018	8.04	0
	A-C		SOLVENT	0246	0015	6.10	0
	ALI		TISSUE	0207	0021	10.14	0
	ALU		TISSUE	0202	0026	12.87	0
	ACP	LI	DMN 90 UM/ML	0255	0483	189.41	0
	ACP	LU	DMN 90 UM/ML	0191	0018	9.42	0
000083885	ACT	LI1	0005-0 PCT.	0217	0015	6.91	0
000083885	ACT	LI2	0025-1 PCT.	0126	0017	13.49	0
000083885	ACT	LI3	0125-2 PCT.	0116	0015	12.93	0
000083885	ACT	LU1	0005-0 PCT.	0172	0017	9.88	0
000083885	ACT	LU2	0025-1 PCT.	0140	0014	10.00	0
000083885	ACT	LU3	0125-2 PCT.	0193	0011	5.70	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 705303 DETECTOR TA1537 SPECIES SPRDAW/RAT DATE - 07/27/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		AMQ 333 UG/ML	0814	0060	7.37	0
	A-C		SOLVENT	0642	0011	1.71	0
	ALI		TISSUE	0593	0021	3.54	0
	ALU		TISSUE	0566	0027	4.77	0
	ACP	LI	AMQ 333 UG/ML	0302	0190	62.91	0
	ACP	LU	AMQ 333 UG/ML	0538	0015	2.79	0
000083885	ACT	LI1	0005-0 PCT.	0478	0013	2.72	0
000083885	ACT	LI2	0025-1 PCT.	0694	0023	3.31	0
000083885	ACT	LI3	0125-2 PCT.	0552	0027	4.89	0
000083885	ACT	LU1	0005-0 PCT.	0552	0023	4.17	0
000083885	ACT	LU2	0025-1 PCT.	0508	0015	2.95	0
000083885	ACT	LU3	0125-2 PCT.	0730	0019	2.60	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 716704 DETECTOR TA1538 SPECIES SPRDAW/RAT DATE - 07/27/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0521	0030	5.76	0
	A-C		SOLVENT	0555	0043	7.75	0
	ALI		TISSUE	0693	0049	7.07	0
	ALU		TISSUE	0684	0051	7.46	0
	ACP	LI	ANTH 67 UG/ML	0684	0868	126.90	0
	ACP	LU	ANTH 67 UG/ML	0578	0048	8.30	0
000083885	ACT	LI1	0005-0 PCT.	0496	0026	5.24	0
000083885	ACT	LI2	0025-1 PCT.	0584	0044	7.53	0
000083885	ACT	LI3	0125-2 PCT.	0618	0042	6.80	0
000083885	ACT	LU1	0005-0 PCT.	0550	0038	6.91	0
000083885	ACT	LU2	0025-1 PCT.	0608	0038	6.25	0
000083885	ACT	LU3	0125-2 PCT.	0634	0044	6.94	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 701204 CONTRACT 223-76-2102 DETECTOR TA98 SPECIES SPRDAW/RAT PROJECT 2672 DATE - 07/27/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0875	0072	8.23	0
	A-C		SOLVENT	1066	0060	5.63	0
	ALI		TISSUE	0663	0269	40.57	0
	ALU		TISSUE	0685	0242	35.33	0
	ACP	LI	ANTH 67 UG/ML	0712	1679	235.81	0
	ACP	LU	ANTH 67 UG/ML	0549	0951	173.22	0
000083885	ACT	LI1	0005-0 PCT.	0646	0085	13.16	0
000083885	ACT	LI2	0025-1 PCT.	0688	0063	9.16	0
000083885	ACT	LI3	0125-2 PCT.	0600	0055	9.17	0
000083885	ACT	LU1	0005-0 PCT.	0657	0061	9.28	0
000083885	ACT	LU2	0025-1 PCT.	1009	0081	8.03	0
000083885	ACT	LU3	0125-2 PCT.	0867	0066	7.61	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 706372 DETECTOR 0000D4 SPECIES SPRDAW/RAT DATE - 07/27/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	0874	0116	0072	13.27	8.24	0
	A-C		SOLVENT	0723	0164	0071	22.68	9.82	0
	ALI		TISSUE	0623	0173	0096	27.77	15.41	0
	ALU		TISSUE	0752	0185	0088	24.60	11.70	0
	ACP	LI	DMN 90 UM/ML	0688	0582	0315	84.59	45.78	0
	ACP	LU	DMN 90 UM/ML	0599	0096	0066	16.03	11.02	0
000083885	ACT	LI1	0005-0 PCT.	0698	0090	0053	12.89	7.59	0
000083885	ACT	LI2	0025-1 PCT.	0709	0086	0051	12.13	7.19	0
000083885	ACT	LI3	0125-2 PCT.	0673	0097	0047	14.41	6.98	0
000083885	ACT	LU1	0005-0 PCT.	0610	0088	0033	14.43	5.41	0
000083885	ACT	LU2	0025-1 PCT.	0651	0130	0051	19.97	7.83	0
000083885	ACT	LU3	0125-2 PCT.	0679	0151	0062	22.24	9.13	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 702009 DETECTOR TA100 SPECIES RHESUS/MONKEY DATE - 07/27/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	0490	0349	71.22	0
	A-C		SOLVENT	0854	0210	24.59	0
	ALI		TISSUE	0886	0716	80.81	0
	ALU		TISSUE	0780	0582	74.62	0
	ACP	LI	DMN 90 UM/ML	1113	1931	173.50	0
	ACP	LU	DMN 90 UM/ML	1016	0748	73.62	0
000083885	ACT	LI1	0005-0 PCT.	0691	0497	71.92	0
000083885	ACT	LI2	0025-1 PCT.	1000	0825	82.50	0
000083885	ACT	LI3	0125-2 PCT.	0963	0711	73.83	0
000083885	ACT	LU1	0005-0 PCT.	0910	0586	64.40	0
000083885	ACT	LU2	0025-1 PCT.	1079	0736	68.21	0
000083885	ACT	LU3	0125-2 PCT.	0802	0669	83.42	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 701208 DETECTOR TA1535 SPECIES RHESUS/MONKEY DATE - 07/27/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	0286	0043	15.03	0
	A-C		SOLVENT	0229	0021	9.17	1
	ALI		TISSUE	0356	0049	13.76	0
	ALU		TISSUE	0333	0058	17.42	0
	ACP	LI	DMN 90 UM/ML	0539	0487	90.35	0
	ACP	LU	DMN 90 UM/ML	0391	0050	12.79	1
000083885	ACT	LI1	0005-0 PCT.	0284	0021	7.39	1
000083885	ACT	LI2	0025-1 PCT.	0282	0039	13.83	0
000083885	ACT	LI3	0125-2 PCT.	0413	0062	15.01	2
000083885	ACT	LU1	0005-0 PCT.	0413	0030	7.26	0
000083885	ACT	LU2	0025-1 PCT.	0286	0038	13.29	0
000083885	ACT	LU3	0125-2 PCT.	0379	0035	9.23	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 705501 DETECTOR TA1537 SPECIES RHESUS/MONKEY DATE - 07/27/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	A+C		AMQ 333 UG/ML	0633	0020	3.16	0
	A-C		SOLVENT	0595	0024	4.03	0
	ALI		TISSUE	0546	0031	5.68	0
	ALU		TISSUE	0646	0028	4.33	0
	ACP	LI	AMQ 333 UG/ML	0539	0311	57.70	0
	ACP	LU	AMQ 333 UG/ML	0645	0018	2.79	0
000083885	ACT	LI1	0005-0 PCT.	0582	0021	3.61	0
000083885	ACT	LI2	0025-1 PCT.	0588	0017	2.89	0
000083885	ACT	LI3	0125-2 PCT.	0570	0025	4.39	0
000083885	ACT	LU1	0005-0 PCT.	0476	0016	3.36	0
000083885	ACT	LU2	0025-1 PCT.	0648	0023	3.55	0
000083885	ACT	LU3	0125-2 PCT.	0633	0013	2.05	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 706786 DETECTOR TA1538 SPECIES RHESUS/MONKEY DATE - 07/27/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		ANTH 67 UG/ML	1571	0028	1.78	0
	A-C		SOLVENT	1483	0041	2.76	0
	ALI		TISSUE	0839	0042	5.01	0
	ALU		TISSUE	0701	0059	8.42	0
	ACP	LI	ANTH 67 UG/ML	1003	1145	114.16	0
	ACP	LU	ANTH 67 UG/ML	0439	0022	5.01	0
000083885	ACT	LI1	0005-0 PCT.	0638	0020	3.13	0
000083885	ACT	LI2	0025-1 PCT.	0602	0022	3.65	0
000083885	ACT	LI3	0125-2 PCT.	0534	0025	4.68	0
000083885	ACT	LU1	0005-0 PCT.	0622	0026	4.18	0
000083885	ACT	LU2	0025-1 PCT.	0572	0032	5.59	0
000083885	ACT	LU3	0125-2 PCT.	0570	0028	4.91	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 701209 DETECTOR TA98 SPECIES RHESUS/MONKEY DATE - 07/27/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0841	0067	7.97	0
	A-C		SOLVENT	0831	0072	8.66	0
	ALI		TISSUE	0545	0169	31.01	0
	ALU		TISSUE	2078	0348	16.75	0
	ACP	LI	ANTH 67 UG/ML	1293	8694	672.39	0
	ACP	LU	ANTH 67 UG/ML	0553	0048	8.68	0
000083885	ACT	LI1	0005-0 PCT.	0854	0041	4.80	0
000083885	ACT	LI2	0025-1 PCT.	1161	0064	5.51	0
000083885	ACT	LI3	0125-2 PCT.	0923	0061	6.61	0
000083885	ACT	LU1	0005-0 PCT.	0964	0048	4.98	0
000083885	ACT	LU2	0025-1 PCT.	0916	0046	5.02	0
000083885	ACT	LU3	0125-2 PCT.	1139	0052	4.57	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 705603 DETECTOR 0000D4 SPECIES RHESUS/MONKEY DATE - 07/27/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	0783	0162	0065	20.69	8.30	0
	A-C		SOLVENT	0766	0133	0059	17.36	7.70	0
	ALI		TISSUE	1034	0163	0095	15.76	9.19	0
	ALU		TISSUE	1130	0233	0101	20.62	8.94	0
	ACP	LI	DMN 90 UM/ML	0669	0591	0418	88.34	62.48	0
	ACP	LU	DMN 90 UM/ML	0643	0104	0077	16.17	11.98	0
000083885	ACT	LI1	0005-0 PCT.	1017	0197	0111	19.37	10.91	0
000083885	ACT	LI2	0025-1 PCT.	0833	0173	0089	20.77	10.68	0
000083885	ACT	LI3	0125-2 PCT.	0763	0123	0084	16.12	11.01	0
000083885	ACT	LU1	0005-0 PCT.	0782	0120	0077	15.35	9.85	0
000083885	ACT	LU2	0025-1 PCT.	1039	0153	0105	14.73	10.11	0
000083885	ACT	LU3	0125-2 PCT.	0967	0148	0094	15.31	9.72	0